receptor dimer and that ligand binding modifies domain mobilities intrinsic to the receptor structure, allowing it to sample a separate, active conformation mediated by network formation.

Membrane Protein Structure & Function I

316-Pos Board B85
Complete and Reversible Chemical Unfolding of an α-Helical Membrane Protein
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The question of how an unordered polypeptide chain assumes its native, biologically active conformation is one of the greatest challenges in molecular biophysics and cell biology. This is particularly true for membrane proteins. Chemical denaturants such as urea have been used successfully for in vitro folding studies of soluble proteins and β-barrel membrane proteins. In stark contrast with these two protein classes, in vitro unfolding of α-helical membrane proteins by urea is often irreversible, and alternative denaturation assays using the harsh detergent sodium dodecyl sulphate suffer from the lack of a common reference state. In line with this, we have recently demonstrated by NMR, CD, and fluorescence spectroscopy that urea is not able to completely abolish the secondary and tertiary structure of the α-helical membrane protein Mistic as long as the protein is solubilised in LDAO micelles. However, now we present the complete and reversible chemical unfolding of Mistic out of alkyl maltoside, cycloalkyl maltoside, and alkyl glucoside micelles. As revealed by automated CD spectroscopy and techniques typically used in β-barrel membrane protein unfolding, Mistic unfolds reversibly following a two-state equilibrium that exhibits the same unfolded reference state irrespective of the detergent used to solubilise the folded protein. The unfolded reference state contains virtually no secondary structure and tertiary contacts. This allows for a direct comparison of the folding energetics in different membrane-mimetic systems and contributes to our understanding of how α-helical membrane proteins fold as compared with β-barrel membrane proteins and water-soluble proteins.

317-Pos Board B86
The 3-4 Loop of Glt_{Ph}, an Archaeal Glutamate Transporter Homolog Mediates the Movement of the Transport Domain
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Excitatory amino acid transporters (EAATs) are a family of secondary transporters responsible for the cessation of glutamatergic synaptic transmission; these proteins catalyze the Na^+ gradient-dependent transport of glutamate out of the synaptic cleft. Recent structural studies of the archaeal EAAT homolog, Glt_{Ph} (which transports aspartate) suggest that transport is achieved by a rigid body, piston-like movement of the transport domain, which houses the substrate binding site in its entirety, across the membrane. The transport domain is connected to an immobile scaffold by 3 loops, one of which, the 3-4 loop, undergoes a substrate sensitive conformational change. Proteolytic cleavage of the 3-4 loop was found to abolish transport activity indicating an essential function for this loop in the transport mechanism. In this work, we demonstrate that despite cleavage of the 3-4 loop, Glt_{Ph} is still able to sample transport relevant conformations. Optimization of the reconstitution conditions reveal that fully cleaved Glt_{Ph} can catalyze some residual transport. Analysis of the kinetics and temperature dependence reveals that this decreased transport activity is not due to alteration of the substrate binding characteristics, but is caused by significantly reduced turnover rate. By measuring crosslink formation rates and solute counterflow activity, we demonstrate that cleaving the 3-4 loop severely compromises the transition of the transport domain between the inward- and outward-facing conformational states. These results reveal a hitherto unknown role for the 3-4 loop in a fundamental step in the transport mechanism.

318-Pos Board B87
Quantitative Characterization of Membrane Deformation by Syndapin (pacsin) and its Autoinhibition
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Membrane curvature (MC) is an important feature of biological membranes in both static and dynamic aspects of cellular functions. However, the molecular machineries for MC sensing and generation are not thoroughly understood. Syndapin (pacsin) is an FBAR domain containing peripheral membrane protein, which participates in different endocytosis pathways and cytoskeletal interactions. The full length syndapin is known to lack membrane deformation ability in the absence of SH3 domain binding partners. This inhibition of membrane tubulation proposed due to an intramolecular BAR/SH3 domain clamping mechanism. However, in order to find out how exactly the SH3 domain interaction perturbs the capacity to deform the membrane requires more quantitative investigation.
We characterize the role of autoinhibition in syndapin MC sensing and generation regulation, using an SH3 domain binding ligand believed to be able to release this autoinhibition. Our major experimental approach is pulling highly bent membrane tethers from pipette-aspirated giant unilamellar vesicles (GUVs). We monitored protein partitioning in the curvature gradient as well as mechanical effects on the membrane through protein binding. With low syndapin concentration, and excess ligand, we observe protein curvature sorting but no curvature generation. With intermediate syndapin concentration, the protein with bound ligand could sense membrane curvature and lower membrane rigidity. When syndapin concentration is high, polymerization of released syndapin on the tethers likely occurs. This research aims to provide thermodynamic explanations to the mechanism of autoinihition regulation of syndapin MC sensing and generation.

319-Pos Board B88
Transporter BetP - Significance of Oligomeric Structure for Catalysis and Regulation
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The Na^+ coupled betaine uptake system BetP of Corynebacterium glutamicum belongs to the BCCT family of transporters and comprises both a catalytic function (betaine/Na^+ cotransport) and a sensory/regulatory function (responding to osmotic stress). Its 2D (electron crystallography) and 3D structure (X-ray crystallography) has been solved. Within a homooligomeric trimer, each BetP protomer harbours both an N- and a C-terminal domain involved in stimulus sensing and intramolecular signal transduction. Factors known so far contributing to the sensory and regulatory function of BetP are (i) the two terminal domains, (ii) K^+ ions as an osmostress related stimulus, and (iii) interaction with the surrounding membrane. Due to the trimeric structure of BetP, functional crosstalk between the individual protomers was suggested for both functions of the transporter.
We have now investigated in detail the significance of the oligomeric (trimeric) structure of BetP for both transport catalysis (betaine uptake) and sensing/regulation (sensing of osmotic stress and intramolecular signal transduction). For this purpose, we have developed a novel experimental tool, in which each of the three BetP protomers can be addressed individually and its contribution to catalysis and regulation elucidated. Each BetP protomer carries an individual molecular tag as well as mutations in specific functional or regulatory domains of the transporter. Using this tool, we were able to quantify the contribution of oligomerization to the catalytic and the regulatory properties of a secondary carrier for the first time.

320-Pos Board B89
Modulation of the pHLIP Transmembrane Helix Insertion Pathway
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The membrane-associated folding/unfolding of pHLIP® (pH (Low) Insertion Peptide) provides an opportunity to study how sequence variations influence the kinetics and pathway of peptide insertion into bilayers. Here we present the results of steady-state and kinetics investigations of several pHLIP variants with different numbers of charged residues, with attached polar cargoes at the peptide's membrane inserting end, and with three single-Trp variants placed at the beginning, middle and end of the transmembrane helix. Each pHLIP variant exhibits a pH-dependent interaction with a lipid bilayer. While the number of protonatable residues at the inserting end does not affect the ultimate formation of helical structure across a membrane, it correlates with the time for peptide insertion, the number of intermediate states on the folding pathway, and the rates of unfolding and exit. The presence of polar cargoes at the peptide's inserting end leads to the appearance of intermediate states on the insertion pathway. Cargo polarity correlates with a decrease of the insertion rate. We